



## Effects of ramadan lifestyle on lipid profile and oxidative stress markers in adult males

Effects of ramadan lifestyle

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### Abstract

**Aim:** In most Muslim societies the daily routine and lifestyle change markedly during the month of Ramadan because of fasting during daylight hours and the altered day and night rituals. These changes could potentially have significant metabolic effects. The aim of this study was to explore the effects of Ramadan fasting and the resultant lifestyle changes on the biochemical profile and oxidative stress markers in healthy adult males. **Material and Method:** Forty-two healthy male volunteers following their usual Ramadan fasting routine were included in the study. The following serum variables were measured at the beginning and at the end of the month: triglycerides, total cholesterol, HDL cholesterol, non-HDL cholesterol, albumin, total protein, CRP, uric acid, ischemia-modified albumin (IMA), albumin adjusted-IMA (AAIMA), and thiol/disulfide homeostasis. **Results:** Triglycerides, total cholesterol, non-HDL cholesterol, IMA and AAIMA levels were significantly higher on the last day of Ramadan compared to the first day ( $p<0.05$ ). HDL cholesterol levels were statistically lower on the last day of Ramadan than the first day ( $p<0.05$ ). However, there were no significant changes in the remaining variables (total albumin, uric acid, CRP, and thiol/disulfide homeostasis parameters). **Discussion:** Lifestyle, nutritional, and diurnal rhythm changes during the month of Ramadan may be associated with hormonal and biorhythm alterations which could be responsible for the observed elevations in serum IMA, AAIMA and non-HDL cholesterol levels. However, further studies are required to ascertain the direct causes of these changes.

### Keywords

Ramadan Fasting; Oxidative Stress; Ischemia Modified Albumin; Thiol/disulfide Homeostasis; Non-HDL Cholesterol

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## Introduction

Fasting is defined as the voluntary abstinence, for a limited period, from solid food, calorie-containing fluids, smoking, and stimulants such as coffee or tea, and it usually implies some degree of caloric restriction (negative daily energy balance). Fasting is one of the 5 pillars of Islam. The type of fasting practiced by Muslims during the month of Ramadan calls for abstaining from eating, drinking, smoking, and engaging in sexual activity during the hours between sunrise and sunset. However, it does not necessarily require maintaining a negative energy balance through caloric restriction. Muslims follow the lunar calendar in which the month is approximately 29.50 days. Every year the month of Ramadan falls approximately 10 days earlier than the previous year. The interval of fasting extends from approximately 1 hour before sunrise to sunset and its length depends on the season in which Ramadan occurs. For instance, during the summer in some parts of the world, fasting may exceed 17 hours [1,2]. Muslims break their fast shortly after sunset with the main meal called “Iftar”, which is often a calorie-rich multi-course affair. Also, just before “Imsak” (the point at which fasting begins 1 hour before sunrise), a light breakfast-like meal (Sahur) is consumed. In addition, it is common for people to stay up late at night visiting relatives and friends, socializing, and indulging in the consumption of food especially sweets, tea, and coffee.

Fasting for a period of weeks at certain times of the year is a common tradition in many cultures since the dawn of civilization, and it is believed to be good for the individual’s body and soul. For this reason, many studies have reported that the potential health benefits of fasting are the subject of laboratory and clinical research [3]. A few randomized controlled trials and observational clinical outcomes studies suggest that fasting is beneficial to health including improved insulin sensitivity, lower fasting insulin levels, and reduced mass of visceral adipose tissue [4,5]. There is also observational evidence that a few weeks of medically supervised fasting (with calorie intake limited to 250-500 kcal per day) is helpful in the treatment of the metabolic syndrome, hypertension, rheumatic diseases, and chronic pain syndromes. Intermittent fasting with caloric restriction may also help to slow the progression of chronic inflammatory diseases [6,7]. However, extensive research in humans is required before recommending fasting as a therapeutic modality. The yearly Muslim fasting ritual presents one opportunity to engage in such research [8-10].

During the month of Ramadan, the day and night rituals and lifestyle of the fasting individual are markedly altered as well as the biorhythm. It is therefore conceivable that during the Ramadan fast significant changes occur in the diurnal rhythm of hormones, substrate flux, and energy metabolism, and that these changes may be accompanied by oxidative stress. The latter is defined as an imbalance between the cellular production of reactive oxygen species (ROS) and the capacity of the antioxidant mechanisms to prevent oxidative damage [11]. Several studies have evaluated the effect of Ramadan fasting on proinflammatory mediators and oxidative stress using a variety of redox markers [12-19]. Al-Shafei and co-workers, using glutathione (GTH) as a measure, reported that Ramadan fasting ameliorated oxidative stress in hypertensive patients [13]. In the present

study, we chose to use the serum levels of ischemia-modified albumin (IMA) and thiol/disulfide homeostasis species to assess oxidative stress in healthy, fasting individuals. To our knowledge, there are no published data using these measures in the context of Ramadan fasting.

## Material and Methods

### Subjects

The study was conducted during Ramadan in June 2016. Forty-two healthy male volunteers aged 25-55 years who were continuing their regular fasting during Ramadan were included in the study. Volunteers with any acute or chronic disease or medication or any addiction except smoking during the study were excluded from the study.

### Sample Preparation for Biochemical Analyses

Two venous blood samples were collected from each volunteer, one on the first day and one on the last day of Ramadan. The samples were collected in the morning into tubes containing gel and centrifuged at 1500 g for 10 min to separate the serums, which were immediately frozen and stored at -80°C until the day of analysis. Hemolyzed and icteric samples were discarded. The samples collected on the first day are considered as the control group while those collected on the last day represent the experimental or fasting group.

Serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-col), albumin, total protein, C-reactive protein (CRP) and uric acid (UA) levels were measured by enzymatic colorimetric methods in a C8000 Architect Abbott auto-analyzer (Rungis, France) using original commercial kits. Non-HDL cholesterol was calculated as the difference between TC and HDL cholesterol.

### Ischemia-modified albumin assay

IMA was measured by a colorimetric assay developed by Bar-Or et al. based on measurement of unbound cobalt after incubation with patient serum [20]. Increased amounts of IMA results in less cobalt binding and more residual unbound cobalt available for complex with a chromogen [dithiothreitol (DTT)], which can be measured photometrically. The procedure was as follows: 50 µL of 0.1% cobalt chloride (Merck KGaA, Darmstadt, Germany) was added to 200 µL of serum, gently mixed, and waited 10 min for adequate cobalt-albumin binding. DTT (50 µL) (Merck KGaA, Darmstadt, Germany), at a concentration of 1.5 mg/ml, was added as a colorizing agent and the reaction was stopped 2 min later by adding 1.0 mL of 0.9% NaCl. The colored product was measured using a spectrophotometer at 470 nm (Shimadzu, UV1601, Japan) and compared to a serum cobalt blank without DTT and reported in absorbance units (ABSU).

The Albumin Adjusted IMA (AAIMA) level was calculated according to the following formula= (Individual serum albumin concentration/ median albumin concentration of the population) x IMA value [21]. Both IMA and AAIMA are still expressed in absorbance units.

### Total and native thiol assay

Serum total and native thiol content was measured using a fully automated colorimetric method developed by Erel et al. [22].

Erel and colleagues used modified Ellman’s reagent to measure the thiol content. For measurement of total thiol, sodium borohydride (NaHB4) was added into sample, so that the dynamic disulfide bonds are reduced to the functional thiol groups. The numberof disulphide bonds in the sample was calculated as (total thiol - native thiol)/2. Inter-essay precisions were 4%, 5% and 13% concentrations of 29.12, 16.03, 7.15 µmol/L respectively. The detection range of the assay was 2.8- 4000 µmol/L.

Statistical analysis

The fasting group (last day samples) and the control group (first-day samples) were compared using SPSS 20.0 (Statistical Package for the Social Sciences). The Kolmogorov–Smirnov test was used to determine whether the parameters were normally distributed. The results were expressed as means ± standard deviations or median and minimum-maximum. Wilcoxon and paired t-test were used for data analysis. A p-value of less than 0.05 was considered statistically significant.

Results

Table 1 shows the serum levels of TG, TC, HDL cholesterol, non-HDL cholesterol, IMA, AAIMA, total protein, CRP, UA, total thiol, native thiol, disulfide, and disulfide/native thiol in the two groups. The serum levels of TG, TC, and non-HDL cholesterol were significantly higher on the last day of Ramadan than on the first day (p < 0.05), whereas the HDL cholesterol levels were significantly lower (p < 0.001). IMA and AAIMA levels were significantly higher on the last day of Ramadan (p < 0.05). Figure 1 shows the changes in results between two groups. There were no significant changes in serum albumin, total protein, CRP, UA, total thiol, native thiol and disulfide, disulfide/native thiol levels (p > 0.05).

Discussion

Metabolic adaptation to hunger is very important for survival. The adaptation of transition from satiety to hunger is well regulated in the human body. The hunger arising from the nature of living is called physiological hunger. The hunger between meals and during sleep is an example of physiological hunger. The post-prandial 2-4 hours is called the absorptive phase and it’s a phase of satiety. For a fasting person, this period starts after “iftar”. In this period levels of glucose, amino acids and TG in blood are raised and insulin/glucagon ratio is also increased. High postprandial insulin levels enhance the entry of glucose into muscle, liver and fat tissues. Glycogen synthesis is stimulated in the muscles and liver, while in fat tissue glucose serves as glycerol-3-phosphate source to build TG. Postprandial biochemical activities are generally an anabolic process which aims to store fuels for the body. Hunger phase starts 2-4 hours after the meals. For a fasting person, this phase starts after “sahur”. This is a catabolic phase as insulin/glucagon ratio is decreased. Starving phase can be divided into two: an early phase (post-absorptive) and prolonged starving. First 6-12 hours is the early phase, when blood glucose is obtained from liver stocks. After 12 hours, the prolonged starving starts and depleted blood glycogen is compensated from other sources like amino acids with gluconeogenesis [23]. In daily life, anabolic phase is observed in days and catabolic phase at nights but

Table 1. Comparison of laboratory findings between two groups

	First day of Ramadan	Last day of Ramadan	p Value
Triglyceride (mg/dL)	117.5 (46-426)	161.5 (56-596)	0.001*
Total cholesterol (mg/dL)	185±37.8	195±39.9	0.005*
HDL cholesterol (mg/dL)	48.6±7.5	44.8±6.3	0.001*
Non-HDL cholesterol (mg/dL)	136.4±37.3	150.1±39.1	0.001*
IMA (ABSU)	0.324±0.07	0.357±0.08	0.020*
Albumin-adjusted IMA (ABSU)	0.319±0.06	0.354±0.07	0.007*
Albumin (g/dl)	4.48±0.21	4.49±0.20	0.579
Total protein (mg/dL)	7.0±0.40	7.12±0.32	0.291
CRP (mg/dL)	0.18 (0.01-1.58)	0.19 (0.02-1.06)	0.586
Uric acid (mg/dL)	6.11±1.04	5.9±0.92	0.095
Total thiol (µmol/L)	409.1±55.2	416.4±60.4	0.386
Native thiol (µmol/L)	373.31±38.80	386.01±47.04	0.074
Disulfide (µmol/L)	18.2 ±7.7	18.7±8.2	0.782
Disulphide/nativethiol; %	4.77±1.94	4.99±2.41	0.750

\*p-value less than 0.05

ABSU - absorbance unit, IMA - ischemia modified albumin, HDL- high density lipoprotein, CRP-C reactive protein

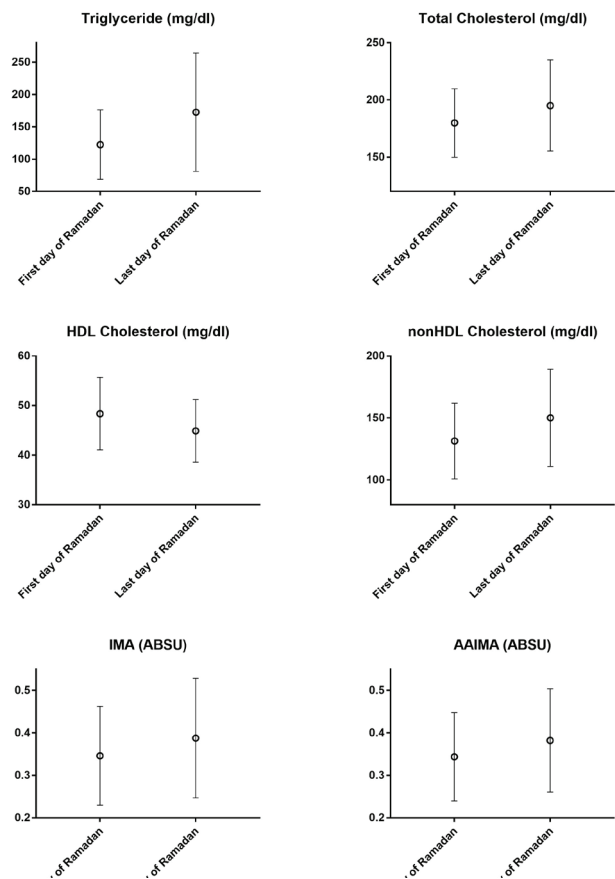


Figure 1. in Ramadan, vice versa occurs. This causes the inverse metabolic cycle. Given these aspects, fasting in Ramadan changes the biorhythm of the body. For that reason, it is expected for the body to change its energy sources and hormone balance [24]. The human metabolism takes all these changes as stress [10]. Several clinical studies reported the effect of Ramadan fasting on oxidative stress [12-19]. However, the studies have measured different biomarkers related to oxidative stress to explain

the effects of Ramadan fasting on oxidative stress. The current study characterizes the effects of Ramadan fasting on serum TG, TC, HDL-col, non-HDL cholesterol, albumin, total protein, CRP, UA, IMA, AAIMA and thiol/disulfide homeostasis parameters (total thiol, native thiol and disulfide) in adult males.

In this study, we report that serum triglyceride and total cholesterol levels were significantly higher on the last day of Ramadan than on the first day. The elevations in triglyceride could be attributed to the increased Glycogen synthesis. Glycogen synthesis stimulates production of glycerol-3-phosphate source to build triglycerides. Also, the elevations in triglyceride and total cholesterol levels in this study could be attributed to blood sampling time. All blood samples were taken in the morning hours. However, several studies reported that the cortisol levels in morning hours during Ramadan are lower than at the same time before Ramadan [25,26]. These findings suggest that cortisol rhythm can be altered during Ramadan [27]. During Ramadan, many people change their sleeping habits and stay awake most of the night. For this reason, it would have been better if the blood samples in this study were taken in the noon hours on the last day of Ramadan.

We report that serum HDL-col levels were significantly lower on the last day of Ramadan than on the first day. And the decrease in HDL-col levels in this study could be attributed to inactivity. It should be noted that Muslims do not fast for the whole day during the month of Ramadan; their activity reduces until sunset. There are studies in the literature evaluating lipid panel and related diseases during Ramadan fasting. But the results of these studies differ from one another. In some studies serum triglyceride, total cholesterol, LDL levels were found to be decreased and HDL-col levels elevated [28-30]. But in other studies, there was no change in lipid profile [31,32]. It seems that the effect of Ramadan fasting on serum lipid levels may be closely related to the nutritional diet and the duration of fasting.

The current study reports that Ramadan fasting increases IMA and significantly adjusted IMA levels in healthy individuals. However, there was a significant increase in non-HDL cholesterol which is an index of risk associated with dyslipidemia [33]. There were also increases in thiol/disulfide homeostasis parameters, but the differences were not significant. This study confirms that oxidative stress in healthy individuals is seen to be higher during Ramadan fasting and also this stress has negative effects with regard to their non-HDL cholesterol levels. These results are generally consistent with the findings of the few studies reporting oxidative stress during fasting [16,17]. The several studies have shown no significant effect on oxidative stress during Ramadan fasting [18,19]. However, other studies reported decreased oxidative stress during Ramadan fasting [12-15]. In a study with 32 healthy participants, triglyceride, total cholesterol and LDL levels were found to be lower during Ramadan, but the HDL-col and a heat-shock protein HSP-70 levels were found to be elevated [15]. Results of another study demonstrated that Ramadan fasting has some positive effects on IL-6, CRP and homocysteine in healthy volunteers [12]. In another study, significant reductions in levels of MDA and the significant elevations in levels of GSH were seen in hypertensive patients during Ramadan [13]. In a study carried out in 27 PCOS patients, beneficial effects on NO and GSH levels were observed [14].

Oxidative status is described as a balance between the development and inactivation of ROS [11]. Any increase in the rate of ROS development, or decrease in their inactivation, may disrupt this balance, resulting in oxidative damage. IMA is a biomarker, which is formed as a consequence of modification of albumin by ROS. Consequently, the elevations in ROS may modify the N-terminal portion of albumin causing an increased formation of IMA. These different levels of IMA in the serum may be used as a marker of the oxidative stress [34].

The thiols are mainly organic compounds having a sulfhydryl group, and cysteine residues, which are functional sulfhydryl groups, are present in many proteins in the human body. Proteins are one of the main targets for oxidative damage. Thiols react with ROS to produce many products as reversible disulfides (RSSR), sulfinic acid and thiol radicals. Disulfides are metabolized to thiols in vivo by specific reductase enzymes, such as thioredoxin (Trx) and glutaredoxin (Grx), to maintain thiol/disulfide homeostasis in the body. Thio/disulfide homeostasis may also be referred to as oxidation-antioxidation homeostasis [22,35]. Consequently, thiol/disulfide homeostasis parameters may be used as a marker of oxidative stress.

In conclusion, Ramadan fasting is an intermittent fasting and different from other hunger. During Ramadan, Muslims disrupt their sleep with sahur. Also, their activity and energy intake patterns change during Ramadan. Given these changing regimens, the diurnal profile of hormones which have a circadian rhythm like cortisol may change. Also, with the initiation of Ramadan fasting, insulin levels and therefore the synthesis of anabolic enzymes in response to insulin will be decreased. A switch from anabolic to catabolic phase occurs with the decreased insulin/glucagon ratio. The changes in biorhythm and cortisol rhythm during Ramadan fasting could be the major reason of elevations in oxidative stress markers. To the best of our knowledge, this is the first study ever to report the effects of Ramadan fasting on IMA and thiol/disulfide homeostasis parameters in healthy individuals. It is necessary to carry out further investigations to illustrate these possible mechanisms. It is also required to evaluate in detail the effect of Ramadan fasting on oxidative stress and various parameters with more participants as well.

### Limitation

This study has its own limitations. The low number of participants and the fact that all of them are males makes it different to apply these results to the general population. Female volunteers were not included in the study, because they may be exempt from fasting during Ramadan at their menstrual period. Physical activity records are not obtained from participants. Smoking is not used as exclusion criteria. Food consumption habits are not recorded so the differences in food regimens are unclear. There were no weight measurements of the participants before and after Ramadan.

### Scientific Responsibility Statement

*The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.*

## Animal and human rights statement

**All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.**

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## Conflict of interest

**None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.**

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